Running head: Predictors of mercury in San Francisco Bay forage fish

Ben K. Greenfield

Environmental Health Sciences Division, School of Public Health

University of California-Berkeley

50 University Hall #7360

Berkeley, CA 94720-7360

Tel: 1 510 507 2365

E-mail: bengreenfield@berkeley.edu

Word Count: 7372

Predictors of mercury spatial patterns in San Francisco Bay forage fish

Ben K. Greenfield †‡ [a], Darell G. Slotton §, Katherine H. Harrold †‖

† San Francisco Estuary Institute, 4911 Central Avenue, Richmond, CA 94804

‡ Corresponding Author: Environmental Health Sciences Division, School of Public Health, University of California-Berkeley, 50 University Hall #7360, Berkeley, CA 94720-7360 Tel: 1 510 507 2365

§ Department of Environmental Science and Policy, University of California-Davis, One Shields Avenue, Davis, CA 95616, USA

‖ Current address: Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, CB #7431, Chapel Hill, NC 27599-7431, USA

a. E-mail: bengreenfield@berkeley.edu

ABSTRACT

Pollution reduction efforts should be targeted towards those sources that result in the highest bioaccumulation. For mercury (Hg) in estuaries and other complex water bodies, carefully designed biosentinel monitoring programs can help identify predictors of bioaccumulation and thereby inform management priorities for source reduction. We employed a probabilistic forage fish Hg survey with hypothesis testing in San Francisco Bay. The study goal was to determine what legacy pollution sources, regions, and landscape features were associated with elevated Hg bioaccumulation. Across 99 sites, whole body Hg concentrations in Mississippi silverside (*Menidia audens*) and topsmelt (*Atherinops affinis*) followed a broad spatial gradient, declining with distance from the Guadalupe River (Pearson's r = -0.69 and -0.45), which drains historic mining areas. Topsmelt Hg increased in Central Bay embayment sites (i.e., enclosed sites including channels, creek mouths, marinas, and subembayments) and in sites with historic Hg contaminated sediments, suggesting an influence of legacy industrial contamination. In 2008, silverside Hg was reduced at sites draining wastewater treatment plants. Fish Hg was not related to abundance of surrounding wetland land cover but was elevated in some watersheds draining from historic Hg mining operations. Results indicated both regional and site-specific influences for Hg bioaccumulation in San Francisco Bay, including spatial patterns in legacy contamination, as well as management actions, such as treated wastewater discharge.

Keywords: mercury, prey fish, estuary, biosentinel, bioaccumulation

INTRODUCTION

Mercury (Hg) pollution can adversely impact human and ecosystem health, particularly via aquatic methylmercury (MeHg) contamination. Global Hg concentrations are elevated due to widespread human use and inadvertent release, creating a need for coordinated efforts to curtail Hg release, transport, and exposure [1]. MeHg is highly toxic and bioaccumulative [2], triggering reproductive effects in wildlife [3] and potential developmental and neurological effects to humans [4, 5]. At the regional scale, carefully designed research and monitoring is needed to prioritize MeHg management actions in the presence of multiple spatially-distributed sources.

Comparative studies of MeHg in forage fish (small, short-lived prey fish, consumed by piscivorous wildlife) aid in describing spatiotemporal patterns and explanatory variables for MeHg food web accumulation [6-13]. Benefits of forage fish relative to other biosentinels include integrating across a several month time period, limited ranges in age and diet, and small home ranges [13]. Thus, forage fish are potentially useful indicators of sources of food web MeHg exposure, locations with elevated bioavailable MeHg, and pathways to reduce risk [6-9]. However, probabilistic spatial surveys and hypothesis testing approaches are rarely employed to evaluate forage fish contamination within a single water body.

San Francisco Bay (the Bay) is influenced by Hg watershed loads and sediment deposits from historic mining operations and industrial sources, making it an important system for characterizing and forecasting effects of ecosystem MeHg exposure [14-17]. As in other estuaries, the spatiotemporal dynamics of MeHg concentrations, bioavailability, and bioaccumulation in the Bay are influenced by complex biogeochemical factors, including variable primary productivity and sulfate reduction, in addition to spatial differences in Hg loading [18-21]. Sources targeted for management reduction include Hg mines, stormwater runoff from urban and industrial watersheds, municipal publically owned treatment works (POTWs), drainage from the Central Valley watersheds, and industrial facilities [16, 22]. Historic Bay sediment contamination also contributes Hg to the water column and food web [23-25].

In addition to sources, there are several spatial factors that may influence MeHg bioaccumulation within the Bay. Bay sediment and biota Hg are elevated in proximity to a historic Hg mining district (New Almaden Mining District), in salt ponds and other semi-enclosed embayments, and in interior wetlands [9, 16, 26-28]. Wetlands are frequently sites of MeHg production, and consequently sources to adjacent ecosystems and biota [29-32]. Enclosed environments, channels, and freshwater tributaries are also frequently associated with increased MeHg in water, sediment, and biota [18, 33-36], due to the combined effects of watershed Hg loading, legacy industrial sources, elevated organic carbon deposition, and spatial variation in biota diets [33, 37-40]. Forage fish sampling could indicate whether proximity to wetlands or embayment areas (such as enclosed marinas, backwater sloughs, stream drainages, and natural sub-embayments) predicts differences in biotic MeHg exposure within an estuary.

This study reports Hg spatial patterns in Bay forage fish collected from 99 sites between 2008 and 2010. Unlike many ambient monitoring programs, the study design was hypothesis-based. Monitoring strata were defined, selected, and randomly subsampled to identify what kinds of locations within the Bay exhibit elevated Hg concentrations in forage fish. Since MeHg is the predominant Hg form in these fish [9], Hg analyses is assumed to indicate MeHg. Four questions are addressed: (1) What are the spatial trends in Bay forage fish Hg? (2) Are Hg concentrations elevated in embayments, relative to open water sites within the Bay? (3) Does the extent of fringing wetland habitat correlate with Hg concentrations? (4) Are concentrations elevated at potential Hg source sites, relative to randomly selected sites? In addition to randomly selected sites, four types of source sites were evaluated: sites draining watersheds impacted by historic Hg mining (mine sites), sites draining urbanized and industrial watersheds (industrial watershed sites), sites receiving treated effluent from wastewater treatment facilities (POTW sites), and sites known to have elevated sediment Hg (contaminated sediment sites).

METHODS

Study design and site selection

The study employed a stratified sampling design, intended to evaluate the four study questions based on *a priori* hypotheses (Supplemental Data Text). The sample design included 99 sites collected along the entire shoreline of San Francisco Bay from Lower South Bay to Suisun Bay (Supplemental Data Figure S1). Wetland channels and estuarine tributaries were included but salt ponds and tidal lakes were excluded. Sites were probabilistically selected from this sample frame using a Generalized Random Tessellation Stratified (GRTS) spatially balanced sampling design [41]. Two sample draws were performed: the first was for random locations across the entire Bay shoreline, and the second was from all identified points within the four source categories, treating each category as a stratum.

The random sample draw included two categories (i.e., strata): open water sites (N = 25 sites) and embayment sites (N = 23 sites; Supplemental Data Figure S2). The source sample draw included four categories: Hg mine creeks (N = 4 sites), watersheds draining urban and industrial areas (N = 13), POTW drainages (N = 7), and areas with relatively elevated sediment THg or MeHg (N = 15). For each subcategory, appropriate sampling locations were identified using GIS, literature, and unpublished data (further detailed in Supplemental Data Text). Due to limited sample sizes for POTWs and Hg mine sites, all sites within these categories were sampled. To ensure sufficient coverage of wetland habitats, 12 additional sites adjacent to nearshore wetlands were sampled in 2008, including 6 sites fringing the South Bay and 6 sites fringing San Pablo and Suisun Bays (Supplemental Information). These wetland sites were only included in the analysis of fringing wetland habitat versus fish Hg.

Fish sampling

All fish sampling was performed by beach seine in 2008, 2009, and 2010. To minimize confounding seasonal variation with spatial variation, study analysis was restricted to the fall season (August 27 to November 30 of each year). The target species were topsmelt (*Atherinops affinis*; target total lengths of 60 - 100 mm) and Mississippi silverside (*Menidia audens*; target total lengths of 40 - 80 mm), both of which have been successfully employed in the Bay as Hg biosentinels [9, 12, 25]. Four composites of five individuals each per species were targeted for total Hg at each sampling event. Target composites each included similar-sized individuals, with the composites distributed in ascending 10 mm size windows spanning the overall size range targeted for each species (i.e. for silverside: composite 1, n = 5 at 40 - 50 mm through composite 4, n=5 at 70 - 80 mm).

For the Bella Oaks and Borges Hg mine sites, target species were not available. At these two sites, prickly sculpin (*Cottus asper*, 52 - 100 mm), California roach (*Hesperoleucus symmetricus*, 54 - 82 mm), and three-spined stickleback (*Gasterosteus aculeatus*, 34 - 50 mm) were collected. Like the target species, these are all benthic invertivores previously employed as Hg biosentinels in California [10, 11, 42-44].

Sample preparation and analysis

All fish collection and preparation followed protocols certified by the UC Davis Veterinary School's Institutional Animal Care and Use Committee (IACUC). Fish were measured for total length, rinsed with site water, and sorted into labeled, freezer-grade plastic bags as composites for analysis, field frozen with air excluded and water surrounding, on dry ice, and subsequently transferred to a -20 °C laboratory freezer. Composite whole body fish samples were subsequently thawed, weighed, dried to constant weight at 55 °C, dry weight and percent solids recorded, and ground to a fine homogenous powder. Samples were analyzed for total Hg at the University of California-Davis. Analysis employed standard cold vapor atomic absorption (CVAA) spectrophotometry, using a dedicated Perkin Elmer Flow Injection Hg System (FIMS) with an AS-90 autosampler, following a two stage digestion under pressure at 90 °C in a mixture of concentrated nitric and sulfuric acids with potassium permanganate. Routine analytical QA/QC included a 67% ratio of QA/QC samples, or 20 for every 30 analytical samples, and included blanks, aqueous standards, continuing control standards, standard reference materials with certified levels of Hg, laboratory split samples, matrix spike samples, and matrix spike duplicates. All results met RMP QA protocols and were well within laboratory control limits. All study Hg results are reported on a wet weight basis.

Geospatial data

Geospatial data were developed in ArcGIS v10. The Bay shoreline was partitioned into open water versus embayment site categories based on visual inspection of a Bay shoreline vector file with depth data overlay, and satellite imagery. Inclusion criteria were depth, degree of separation from the rest of the Bay, and presence of channels or sloughs. The enclosed layer included habitats within each sub-embayment, with the largest areal coverage north of San Pablo and Suisun Bays (Supplemental Data Figure S2).

Two numeric geospatial attributes were examined for association with fish Hg: percent surrounding wetland area and distance from the Guadalupe River. Percent surrounding wetland area was based on a 500 m buffer, using data from Bay Area Aquatic Resource Inventory and Association of Bay Area Governments 2005 land-use polygons. Percent surrounding wetland was defined as the sum of the depressional, marsh, and tidal ditch land cover categories. Distance from the Guadalupe River, defined as the nautical distance from the westernmost tidal point of Coyote Creek, was negatively correlated with forage fish Hg at 22 sites sampled previously [9]. It was calculated following along the deep Bay channel, extending from the starting point to the upstream study extent of Suisun Bay (Mallard Island, near the confluence of the Sacramento and San Joaquin Rivers). Distance from the Guadalupe River indicates how close the sites are to the Hg contaminated New Almaden Mining District, which drains into the Lower South Bay near the community of Alviso. However, distance from the Guadalupe River also indicates general position along the Bay axis, with the most distant north Bay segments (Suisun Bay, San Pablo Bay) having potentially different net MeHg production and distribution from the progressively closer Central Bay, South Bay, and Lower South Bay [9, 18].

Data analysis

Data analyses were performed using the linear mixed effects model function in R 2.15 [45]. Separate linear models were built to examine the potential effect of embayment category (embayment versus open Bay), surrounding wetland abundance, or site type (e.g., POTW, contaminated sediment, industrial watershed, and random sites), on topsmelt or silverside Hg. Hg data were $\log_{10}$ transformed to improve residual normality and variance homoskedasticity. Topsmelt site type evaluation compared contaminated sediment and industrial watershed sites to random (open and embayment) sites; POTW sites were not included because topsmelt were only collected at one POTW site. Embayment category evaluation was performed on random sites only, to avoid confounding site type versus embayment category.

Model evaluation was performed manually, using backwards elimination of non-significant model terms, and following the ten step protocol recommended by Zurr et al. [46] (Sections 4.2.3 and 5.10) to assess a mixed model approach to nested data. Parameter inclusion was based on the likelihood ratio test (with an alpha=0.05 to retain a parameter) in combination with information theoretic criteria (i.e., AIC and BIC) [47]. In all cases, model fit and residual behavior significantly improved when including a random term for sampling site. Therefore, mixed models were employed, including a random intercept effect for site, and a random slope (i.e., length) effect for site if warranted. The initial model fixed structure always included year terms (2008 and 2009), fish length, distance from the Guadalupe River, and the effect under consideration (embayment category, site type, or surrounding wetland), and one way interaction terms between site effects and the other model terms. For example, the initial model to evaluate embayment effect was: Log(Hg) = Year2008 + Year2009 + FishLength + DistanceGuadalupe + Embayment + Year2008*Embayment + Year2009*Embayment + FishLength*Embayment + DistanceGuadalupe*Embayment.

Of the four mine sites, silverside was only present at Guadalupe River upstream of Alviso Slough and topsmelt was only present at American Canyon Creek, draining Borges Mine. Since this was insufficient to statistically evaluate a mine site effect for these species, each mine site was compared to other data reported for additional species on an *ad hoc* basis. To provide context, data on additional species were compared to previously published Hg concentration data from mine sites [10, 43, 44] and unpublished data from reference (i.e., no known mine influence) sites. Unpublished data were obtained via queries performed on March 23, 2013 of the California Environmental Data Exchange Network (www.ceden.us), a collaboratively developed statewide environmental water quality database [48].

RESULTS

Graphical analysis indicated a spatial trend in average forage fish Hg concentrations, with the highest concentrations in and adjacent to Lower South Bay, and concentrations progressively decreasing towards South, Central, San Pablo, and Suisun Bays (Figure 1). This spatial gradient was more pronounced for silverside (Figure 1a), whereas topsmelt exhibited more local scale spatial heterogeneity, particularly within Central Bay (Figure 1b).

Hg concentrations were higher in silverside (0.091 ± 0.059 μg/g, mean ± SD, N = 240) than topsmelt (0.042 ± 0.020, N = 240), as reported in a prior study [9]. For silversides, the Cooley Landing site (N = 3, located west of Lower South Bay) had extreme variance heterogeneity due to only having one individual per composite, which caused outliers and violation of normality in residuals. For topsmelt, one of the composite samples collected in Alviso Slough (south of Lower South Bay) in 2009 was an outlier (standardized residual = 4.68), with an Hg concentration of 0.235 μg/g, versus a range of 0.015 to 0.114 μg/g for the remaining 239 samples. Remaining results are reported excluding these outlier samples, but were essentially unchanged when they were included.

Total length was positively related to Hg and included as a covariate in all models. Sampling year differences (treated as a categorical variable) were significant and included in some models (Table 1). Mixed models were needed to account for correlations among samples within a site. For silverside and for examination of wetland effects in topsmelt, a random intercept and random slope effect on length were incorporated; for remaining topsmelt models, only a random intercept term was needed.

Distance from Guadalupe River

Distance from the Guadalupe River (Question 1) was negatively related to Hg in silverside (r = -0.69, N = 237, Figure 1a) and topsmelt (r = - 0.45, N = 232, Figure 1b). Distance was also a significant predictor in mixed models accounting for site effect ($p < 0.0001$, Table 1), and was therefore included as a covariate in models testing for other effects. The final models for silverside indicated that in 2009, the decrease in Hg with distance from the Guadalupe River was weaker than other years (DistanceGuadalupe*Year2009 interaction, Figure 2). Based on model predicted concentrations, in 2009, the closest site to the Guadalupe River (Coyote Creek near San Jose) exhibited two-fold higher Hg concentrations than the furthest site (Kirker Creek near Pittsburg; 0.112 vs. 0.053 μg/g), whereas in 2010, the predicted difference was four-fold (0.156 vs. 0.039 μg/g).

Embayment and fringing wetland effects

Embayment versus open water (Question 2) was not significant (likelihood ratio test p = 0.096) for silverside (Supplemental Table S2). However, for topsmelt, embayment sites were elevated in Hg (p = 0.012), and embayment site Hg significantly increased with distance from the Guadalupe River and with fish length (Table 1, Supplemental Table S3). Embayment sites in Central and San Pablo Bays were more often elevated in topsmelt Hg versus adjacent open water sites (Figs. 1b, 3). For example, at the embayment site furthest from the Guadalupe River (the Petaluma River site), model predicted topsmelt Hg would be 0.043 μg/g, whereas an open site at the same distance would have a predicted Hg of 0.029 μg/g.

Percent surrounding wetlands (Question 3) was not a significant predictor of Hg for silverside or topsmelt. For both species, the final model included a significant increase with body length, a significant decrease with distance from the Guadalupe River, and no effect of wetlands (Table 1, Supplemental Tables S4 and S5). For silverside, the high wetland sites were in channels surrounding San Pablo and Suisun Bays, and had lower Hg than Lower South Bay and South Bay sites (Figure 1a).

Source site type effects

Source site effects (Question 4) varied between silverside and topsmelt. In 2008, silverside Hg was lower at POTW sites than other site types (SourcePOTW*Year2008 interaction, Figure 2, Supplemental Table S6). Based on model predictions for average length fish, in 2008, POTW sites had about one half of the Hg of non-POTW sites (0.035 vs. 0.068 μg/g); in 2009 and 2010, POTW sites were predicted to be 0.015 μg/g lower than non-POTW sites. Graphical analysis indicated POTW sites to be lower than nearby sites in both 2008 and 2010 (Figure 2). In 2009, there was no apparent pattern of POTW versus other sites. Topsmelt were only obtained at one POTW site, the Hayward wastewater treatment plant discharge pond, monitored in 2010. Topsmelt Hg concentrations at that site (0.021 ± 0.0005 μg/g, N = 4) were less than half the concentrations at the nearest site measured in 2010, the Eden Landing Shoreline (0.045 ± 0.007 μg/g, N = 4).

Hg in topsmelt was moderately elevated for contaminated sediment sites ($p = 0.032$, Table 1, Supplemental Table S7), which were only present in Lower South, South, and Central Bays (Figure 4). The model predicted topsmelt Hg at a contaminated sediment site to be 1.2 times that predicted for another site type in the same location. In 2008, the overall decrease with distance from the Guadalupe River was weaker (Year2008*DistanceGuadalupe).

Fish species captured varied across the mining sites (Table 2), likely due to variable salinity conditions. The Guadalupe River upstream of Alviso Slough, which drains from the New Almaden Mine watershed, was elevated in silverside Hg, consistent with the general spatial gradient observed in this study and elsewhere [9, 16, 17, 25]. Concentrations at this site were within the range of spatial variation observed in Lower South Bay (Figure 1a), but higher than the Baywide average and silverside from Hg contaminated Clear Lake [10]. The Guadalupe River site also had extremely high Hg in three-spined stickleback. In the Napa River below the Bella Oaks Mine watershed, prickly sculpin Hg was comparable to Clear Lake [10] and higher than the average of eight sites from the Sacramento-San Joaquin Rivers Delta. Napa River California roach Hg was higher than the average of eight CA statewide sites lacking mine influence, but well below the concentration previously measured by Slotton et al. [43] at Marsh Creek, a mine dominated creek that drains into Suisun Bay. American Canyon Creek, which drains from the Borges mine, was not elevated in topsmelt Hg, relative to general Baywide concentrations. Dry Creek also had relatively low Hg concentrations in prickly sculpin and California roach, and unremarkable concentrations in three-spined stickleback, suggesting a lack of influence of the nearby La Joya mine.

Three-spined stickleback were relatively low in Hg within the urbanized industrial watershed of Zone 4 Line A, compared to mine sites and four other Bay sites. The Zone 4 Line A sampling location is within a flood drainage channel, several km above the Bay shoreline [49].

DISCUSSION

Mercury concentrations were found to be elevated in the southern Bay in forage fish in this study and previously [9], as well as for southern Bay water, sediments, and shorebirds [16, 18, 50], suggesting that greater attention be dedicated to MeHg management in this region. The higher Hg concentrations in Lower South and South Bay likely result from multiple factors including: South Bay hydrodynamics, historical Hg loading from the New Almaden Hg Mining District, and methylation in the Bay and adjacent habitats. Relatively long water residence times in the Lower South and South Bay may result in reducing conditions that favor sediment and water column MeHg production [16], with additional elevated MeHg production in the extensive salt pond complexes adjacent to Lower South Bay [27, 28], and periodic anoxia along Alviso Slough itself [12]. The importance of distance to the Guadalupe River for Hg concentrations suggests the importance of broad spatial gradients, which may be increased due to tidal mixing and small scale fish movements.

Elevated Hg in topsmelt but not silverside at embayment sites (e.g., marinas, creeks, and backwater sloughs), and no relationship between surrounding wetlands and fish Hg, suggest a limited ability to predict biotic MeHg exposure based on natural landscape attributes. We hypothesized that surrounding wetland abundance would correlate with forage fish Hg based on the established role of freshwater wetlands as MeHg sources to adjacent waters [30], the consequent association between proximity to wetlands and freshwater fish Hg [8, 31, 32], and evidence of elevated MeHg production in estuarine wetland sediment [26, 29, 51]. Despite this, no association was observed, suggesting that MeHg bioaccumulation in Bay forage fish is decoupled from fringing wetlands, with other factors driving Bay bioaccumulation patterns.

Our hypothesis that embayment status could predict increased MeHg exposure in forage fish was based on elevated Hg accumulation in Bay forage fish species that heavily utilize intertidal and shoreline areas (e.g., silverside) [9], elevated sediment and biota MeHg in proximity to freshwaters in the Bay and other estuaries [18, 33-36], increased exposure to anthropogenic Hg pollution at embayment sites [27, 40] and the possible importance of fringing wetlands, intertidal mudflat habitat, and shallow sediments for MeHg production at embayment sites [26, 34, 51]. The increase in topsmelt Hg from embayment sites was related to spatial location; differences were primarily observed in Central Bay, where silversides were not readily available. We speculate that the embayment pattern for topsmelt largely stems from exposure to historic industrial contamination, because topsmelt Hg was also increased near legacy contaminated sediment in this study. Historic industrial activity was abundant in Central Bay shoreline, and is associated with elevated concentrations of PCBs, another legacy and industrial pollutant, in sediment and forage fish [52, 53]. This pattern suggests that regional priorities for minimizing MeHg production might focus on identifying and restoring those embayment sites with elevated sediment and biota MeHg.

Source site type effects included higher topsmelt Hg near contaminated sediments, higher Hg near some historic mine drainages, and lower Hg adjacent to POTWs in 2008 and possibly 2010. Previous research suggests that Bay forage fish Hg and PCBs are sediment derived [25, 53], and in this study, topsmelt but not silverside Hg exhibited an association with contaminated sediment. Other studies have also exhibited variable relationships between fish and sediment Hg (or MeHg), with associations observed in Texas rivers [7], the Hudson River (New York/New Jersey) [54], and the Willamette River (Oregon/Washington) [55], but not in a survey of northeastern US freshwaters [56] or a Columbia River (Washington) reservoir [57]. In the Gulf of Maine, biota Hg is generally elevated in regions with elevated sediment Hg, but the bioaccumulation factor is lower in more contaminated areas, due to elevated total organic carbon reducing bioavailability [39]. The complexity of Hg methylation and bioavailability, biota movement, and food web structure all contribute to the weak and variable relationships between fish and sediment Hg [39, 57, 58].

The negative effect of POTWs on forage fish Hg was unexpected given that average total MeHg detected in discharge water from the 16 largest Bay POTWs was 0.37 ng/L, versus 0.096 ng/L in Bay ambient water [15]. The lower than expected forage fish Hg concentrations at some POTW sites may result from biodilution, as when increased primary and secondary production decreases Hg bioaccumulation and biomagnification [59, 60]. The reduced silverside Hg concentration at four South Bay POTW sites is associated with elevated discharge water ammonium concentrations, compared to ambient Bay conditions [61]. This may result in increased rates of primary production, higher densities of silversides and their invertebrate prey, or more rapid growth rates, all resulting in decreased tissue Hg concentrations.

In this study, local mining impacts were inconsistent, especially compared to the broad spatial gradient across the Bay. Concentrations in proximity to mining-impacted sites varied widely: the Guadalupe River downstream of New Almaden Mining District and the Napa River below the Bella Oaks Mine were elevated in fish Hg, and at or above prior measurements of the same fish species in mine influenced sites [10, 43]. In contrast, American Canyon Creek and Dry Creek were not elevated. In California roach monitored closer to the New Almaden Mines (Guadalupe Creek at Meridian Ave and Alamitos Creek at Harry Road), Hg concentrations were even greater, versus other sites in the local Guadalupe River watershed [62], and Hg isotopes indicate a New Almaden mining source signal in sediment and forage fish [17, 25]. In freshwater lakes and rivers, fish Hg concentrations are frequently elevated in sites impacted by mining waste versus reference sites, and tend to decrease with increasing distance from mining sources [10, 55, 63-65]. Hg is elevated near mines, processing facilities, or waste tailings even in areas with naturally occurring Hg deposits, and even with Hg mining completed several decades before fish collection, indicating a remaining concern for mine Hg in the food web.

Finally, sites adjacent to industrial watersheds hypothesized to be Hg-contaminated did not exhibit elevated forage fish Hg concentrations. This is consistent with the relatively small Hg mass discharged from these industrial watersheds, compared to other sources and Bay sediments. The San Francisco Bay TMDL Staff Report [22] estimates urban stormwater runoff to contribute

92 kg/yr Hg to the Bay, which was only 7.5% of all sources (1222 kg/yr) [22], and Hg isotope studies found a significant relationship between sediments and forage fish, without any notable deviations adjacent to more industrial sites [25]. Even stickleback collected within a small industrial watershed (Zone 4 Line A) were lower than at other sites, suggesting that industrial watersheds are not locations of elevated MeHg bioaccumulation.

This study demonstrated the use of biosentinel forage fish, combined with a stratified probabilistic survey design, to identify Hg bioaccumulation spatial patterns and sources in a single urbanized estuary. Both regional and local patterns were observed, reflecting the complex legacy Hg sources and system hydrology. Regionally, there was a clear spatial gradient with distance from a historic Hg mining district. After accounting for that gradient, local differences among sites were subtle and varied between fish species. These findings suggest that forage fish Hg bioaccumulation predominantly exhibits broad regional variation, and that sources varying at local scales, including POTW-associated biodilution and legacy sediment Hg contamination, exhibit a secondary influence.

SUPPLEMENTAL DATA

Forage fish mercury spatial patterns and predictors in San Francisco Bay (Supplemental text, tables and figures), and field and laboratory Hg data on 1260 forage fish samples (SFForageFishHg.csv).

ACKNOWLEDGEMENTS

N Feger, C Sommers, RE Looker, R Schlipf, and the RMP Technical Review Committee assisted with site selection. SM Ayers, K Ridolfi, AR Melwani, RM Allen, and MJ Lent assisted with field sampling. AR Melwani and J Griswold helped with statistical analysis, and RM Allen and M Klatt helped with data interpretation. JA Davis, DB Senn, MD Sedlak, and M Lahiff constructively reviewed drafts of the manuscript. Site access was provided by CDPR, CDFG, East Bay RPD, Mt. View Sanitary District, USACE, and SF Bay National Wildlife Refuge. This study was funded by the Regional Monitoring Program for Water Quality in San Francisco Bay (SFEI Publication ###), with additional support by a USEPA STAR Fellowship to BG.

REFERENCES

1. Lambert KF, Evers DC, Warner KA, King SL, Selin NE. 2012. Integrating mercury science and policy in the marine context: Challenges and opportunities. *Environ Res* 119:132-142.

2. Wiener JG, Spry DJ. 1996. Toxicological significance of mercury in freshwater fish. In Beyer WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental contaminants in wildlife: interpreting tissue concentrations*. Lewis, Boca Raton, FL, pp 297-339.

3. Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12-18.

4. National Research Council. 2000. *Toxicological effects of methylmercury*. Committee on the Toxicological Effects of Methylmercury. National Academies Press.

5. Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, Cowell W, Grandjean P, Korrick S. 2012. Evidence on the human health effects of low-level methylmercury exposure. *Environ Health Persp* 120:799-806.

6. Snodgrass JW, Jagoe CH, Bryan AL, Brant HA, Burger J. 2000. Effects of trophic status and wetland morphology, hydroperiod, and water chemistry on mercury concentrations in fish. *Can J Fish Aquat Sci* 57:171-180.

7. Smith A, Abuzeineh AA, Chumchal MM, Bonner TH, Nowlin WH. 2010. Mercury contamination of the fish community of a semi-arid and arid river system: Spatial variation and the influence of environmental gradients. *Environ Toxicol Chem* 29:1762-1772.

8. Wiener JG, Knights BC, Sandheinrich MB, Jeremiason JD, Brigham ME, Engstrom DR, Woodruff LG, Cannon WF, Balogh SJ. 2006. Mercury in soils, lakes, and fish in Voyageurs National Park (Minnesota): importance of atmospheric deposition and ecosystem factors. *Environ Sci Technol* 40:6261-6268.

9. Greenfield BK, Jahn A. 2010. Mercury in San Francisco Bay forage fish. *Environ Pollut* 158:2716-2724.

10. Suchanek T, Eagles-Smith C, Slotton D, Harner E, Colwell A, Anderson N, Mullen L, Flanders J, Adam D, McElroy K. 2008. Spatio-temporal trends of mercury in fish from a mine-dominated ecosystem at Clear Lake, California: individual, species and population level trends. *Ecol Appl* 18:A177-A195.

11. Eagles-Smith CA, Ackerman JT. 2009. Rapid changes in small fish mercury concentrations in estuarine wetlands: implications for wildlife risk and monitoring programs. *Environ Sci Technol* 43:8658-8664.

12. Greenfield BK, Melwani AR, Allen RM, Slotton DG, Ayers SM, Harrold KH, Ridolfi K, Jahn A, Grenier JL, Sandheinrich MB. 2013. Seasonal and annual trends in forage fish mercury concentrations, San Francisco Bay. *Sci Total Environ* 444:591-601.

13. Wiener JG, Bodaly RA, Brown SS, Lucotte M, Newman MC, Porcella DB, Reash RJ, Swain EB. 2007. Monitoring and evaluating trends in methylmercury accumulation in aquatic biota. In Harris R, Krabbenhoft DP, Mason R, Murray MW, Reash R, Saltman T, eds, *Ecosystem*

Responses to Mercury Contamination: Indicators of Change. CRC Press, Pensacola, FL, pp 87-122.

14. Conaway CH, Black FJ, Grieb TM, Roy S, Flegal AR. 2008. Mercury in the San Francisco Estuary: A review. *Rev Environ Contam Toxicol* 194:29-54.
15. Yee D, McKee LJ, Oram JJ. 2011. A regional mass balance of methylmercury in San Francisco Bay, California, USA. *Environ Toxicol Chem* 30:88-96.
16. Davis JA, Looker RE, Yee D, Marvin-Dipasquale M, Grenier JL, Austin CM, McKee LJ, Greenfield BK, Brodberg R, Blum JD. 2012. Reducing methylmercury accumulation in the food webs of San Francisco Bay and its local watershed. *Environ Res* 119:3-26.
17. Gehrke GE, Blum JD, Marvin-DiPasquale M. 2011. Sources of mercury to San Francisco Bay surface sediment as revealed by mercury stable isotopes *Geochim Cosmochim Acta* 75:691-705.
18. Conaway CH, Squire S, Mason RP, Flegal AR. 2003. Mercury speciation in the San Francisco Bay estuary. *Mar Chem* 80:199-225.
19. Marvin-DiPasquale M, Agee JL. 2003. Microbial mercury cycling in sediments of the San Francisco Bay-Delta. *Estuaries* 26:1517-1528.
20. Hammerschmidt CR, Fitzgerald WF. 2004. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ Sci Technol* 38:1487-1495.
21. Luengen A, Flegal AR. 2009. Role of phytoplankton in mercury cycling in the San Francisco Bay estuary. *Limnol Oceanogr* 54:23-40.
22. SFBRWQCB. 2006. Mercury in San Francisco Bay Total Maximum Daily Load (TMDL) Proposed Basin Plan Amendment and Staff Report for Revised Total Maximum Daily Load

(TMDL) and Proposed Mercury Water Quality Objectives. *Final Report.* California Regional Water Quality Control Board San Francisco Bay Region, Oakland, CA

23. Hornberger MI, Luoma SN, van Geen A, Fuller C, Anima R. 1999. Historical trends of metals in the sediments of San Francisco Bay, California. *Mar Chem* 64:39-55.
24. Conaway CH, Watson EB, Flanders JR, Flegal AR. 2004. Mercury deposition in a tidal marsh of south San Francisco Bay downstream of the historic New Almaden mining district, California. *Mar Chem* 90:175-184.
25. Gehrke GE, Blum JD, Slotton DG, Greenfield BK. 2011. Mercury isotopes link mercury in San Francisco Bay forage fish to surface sediments. *Environ Sci Technol* 45:1264-1270.
26. Heim WA, Coale KH, Stephenson M, Choe K-Y, Gill GA, Foe C. 2007. Spatial and habitat-based variations in total and methyl mercury concentrations in surficial sediments in the San Francisco Bay-Delta. *Environ Sci Technol* 41:3501-3507.
27. Miles AK, Ricca MA. 2010. Temporal and spatial distributions of sediment mercury at salt pond wetland restoration sites, San Francisco Bay, CA, USA. *Sci Total Environ* 408:1154-1165.
28. Grenier JL, Davis JA. 2010. Water quality in South San Francisco Bay, California: current condition and potential issues for the South Bay Salt Pond Restoration Project. *Rev Environ Contam Toxicol* 206:115-147.
29. Bergamaschi BA, Fleck JA, Downing BD, Boss E, Pellerin B, Ganju NK, Schoellhamer DH, Byington AA, Heim WA, Stephenson M, Fujii R. 2011. Methyl mercury dynamics in a tidal wetland quantified using in situ optical measurements. *Limnol Oceanogr* 56:1355-1371.
30. Rudd JWM. 1995. Sources of methyl mercury to freshwater ecosystems: a review. *Water Air Soil Pollut* 80:697-713.

31. Chumchal MM, Drenner RW, Fry B, Hambright KD, Newland LW. 2008. Habitat-specific differences in mercury concentration in a top predator from a shallow lake. *T Am Fish Soc* 137:195-208.
32. Chumchal MM, Hambright KD. 2009. Ecological factors regulating mercury contamination of fish from Caddo Lake, Texas, USA. *Environ Toxicol Chem* 28:962-972.
33. Farmer TM, Wright RA, DeVries DR. 2010. Mercury concentration in two estuarine fish populations across a seasonal salinity gradient. *T Am Fish Soc* 139:1896-1912.
34. Fry B, Chumchal MM. 2012. Mercury bioaccumulation in estuarine food webs. *Ecol Appl* 22:606-623.
35. Harris R, Pollman C, Landing W, Evans D, Axelrad D, Hutchinson D, Morey SL, Rumbold D, Dukhovskoy D, Adams DH, Vijayaraghavan K, Holmes C, Atkinson RD, Myers T, Sunderland E. 2012. Mercury in the Gulf of Mexico: Sources to receptors. *Environ Res* 119:42-52.
36. Benoit JM, Gilmour CC, Mason RP, Riedel GS, Riedel GF. 1998. Behavior of mercury in the Patuxent River estuary. *Biogeochemistry* 40:249-265.
37. Turekian KK. 1977. The fate of metals in the oceans. *Geochimica et Cosmochimica Acta* 41:1139-1144.
38. Lambertsson L, Nilsson M. 2006. Organic material: The primary control on mercury methylation and ambient methyl mercury concentrations in estuarine sediments. *Environ Sci Technol* 40:1822-1829.
39. Chen CY, Dionne M, Mayes BM, Ward DM, Sturup S, Jackson BP. 2009. Mercury bioavailability and bioaccumulation in estuarine food webs in the Gulf of Maine. *Environ Sci Technol* 43:1804-1810.

40. Sunderland EM, Amirbahman A, Burgess NM, Dalziel J, Harding G, Jones SH, Kamai E, Karagas MR, Shi X, Chen CY. 2012. Mercury sources and fate in the Gulf of Maine. *Environ Res* 119:27-41.

41. Stevens DL, Jr., Olsen AR. 2004. Spatially balanced sampling of natural resources. *J Am Stat Assoc* 99:262-278.

42. Moyle PB. 2002. *Inland Fishes of California*. University of California Press, Berkeley, CA.

43. Slotton DG, Ayers SM, Reuter JE. 1996. Marsh Creek Watershed 1995 Mercury Assessment Project Final Report to Contra Costa County, Davis, CA

44. SFBRWQCB. 2008. Total Maximum Daily Load for Mercury in Walker Creek Watershed. California Regional Water Quality Control Board San Francisco Bay Region, Oakland, CA. http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/walkermercury/Staff_Report_Final.pdf

45. R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

46. Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, Berlin, Germany.

47. Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: A practical information-theoretic approach*, 2nd ed. Springer, Berlin, Germany.

48. CEDEN. 2012. California Environmental Data Exchange Network General Information. Fact Sheet. http://www.ceden.org/docs/ceden_intro_010912.pdf

49. McKee LJ, Gilbreath AN, Eads R. 2009. Concentrations and Loads of Trace Contaminants in the Zone 4 Line A Small Tributary, Hayward, California: Water Year 2007. 563. San

Francisco Estuary Institute.
http://www.sfei.org/sites/default/files/563_Z4LA_Year1final4_web.pdf

50. Ackerman JT, Eagles-Smith CA, Takekawa JY, Demers SA, Adelsbach TL, Bluso JD, Miles AK, Warnock N, Suchanek TH, Schwarzbach SE. 2007. Mercury concentrations and space use of pre-breeding American avocets and black-necked stilts in San Francisco Bay. *Sci Total Environ* 384:452-466.
51. Marvin-DiPasquale MC, Agee JL, Bouse RM, Jaffe BE. 2003. Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California. *Environ Geol* 43:260-267.
52. Davis JA, Hetzel F, Oram JJ, McKee LJ. 2007. Polychlorinated biphenyls (PCBs) in San Francisco Bay. *Environ Res* 105:67-86.
53. Greenfield BK, Allen RM. 2013. Polychlorinated biphenyl spatial patterns in San Francisco Bay forage fish. *Chemosphere* 90:1693-1703.
54. Levinton JS, Pochron ST. 2008. Temporal and geographic trends in mercury concentrations in muscle tissue in five species of Hudson River, USA, fishes. *Environ Toxicol Chem* 27:1691-1697.
55. Hope BK, Rubin JR. 2005. Mercury levels and relationships in water, sediment, and fish tissue in the Willamette Basin, Oregon. *Arch Environ Contam Toxicol* 48:367-380.
56. Kamman NC, Chalmers A, Clair TA, Major A, Moore RB, Norton SA, Shanley JB. 2008. Factors influencing mercury in freshwater surface sediments of northeastern North America. *Ecotoxicology* 14:101-111.

57. Munn MD, Short TM. 1997. Spatial heterogeneity of mercury bioaccumulation by walleye in Franklin D. Roosevelt Lake and the Upper Columbia River, Washington. *T Am Fish Soc* 126:477-487.
58. Fowlie AR, Hodson PV, Hickey MBC. 2008. Spatial and seasonal patterns of mercury concentrations in fish from the St. Lawrence River at Cornwall, Ontario: Implications for monitoring. *J Great Lakes Res* 34:72-85.
59. Chen CY, Folt CL. 2005. High plankton densities reduce mercury biomagnification. *Environ Sci Technol* 39:115-121.
60. Essington TE, Houser JN. 2003. The effect of whole-lake nutrient enrichment on mercury concentration in Age-1 yellow perch. *T Am Fish Soc* 132:57-68.
61. Driscoll CT, Chen CY, Hammerschmidt CR, Mason RP, Gilmour CC, Sunderland EM, Greenfield BK, Buckman KL, Lamborg CH. 2012. Nutrient supply and mercury dynamics in marine ecosystems: a conceptual model. *Environ Res* 119:118-131.
62. Tetra Tech Inc. 2005. Guadalupe River Watershed Mercury TMDL Project Technical Memorandum 5.3.2. Data Collection Report. Prepared for Santa Clara Valley Water District, Lafayette, CA
63. Weech SA, Scheuhammer AM, Elliott JE, Cheng KM. 2004. Mercury in fish from the Pinchi Lake Region, British Columbia, Canada. *Environ Pollut* 131:275-286.
64. Leady BS, Gottgens JF. 2001. Mercury accumulation in sediment cores and along food chains in two regions of the Brazilian Pantanal. *Wetlands Ecology and Management* 9:349-361.

65. Gustin MS, Saito L, Peacock M. 2005. Anthropogenic impacts on mercury concentrations and nitrogen and carbon isotope ratios in fish muscle tissue of the Truckee River watershed, Nevada, USA. *Sci Total Environ* 347:282-294.

Table 1. Results of study model evaluations

					Final model	
Test (Questions)[a]	Species	N	Likelihood ratio	p-value	Fixed effects	Random effects
1. Distance from Guadalupe[b] (1)	Silverside	237	44.8	< 0.0001	Distance (-)	Intercept, Length[c]
Distance from Guadalupe (1)	Topsmelt	239	12.5	0.0004	Distance (-)	Intercept, Length
Embayment (2)	Silverside	116	2.77	0.096 (NS)	Distance (-), Length (+)	Intercept, Length
Embayment (2)	Topsmelt	133	6.30	0.012	Distance (-), Length (+), 2009 (+), Embayment (+), Distance*Embayment (+), Length*Embayment (+)	Intercept
Embayment Length Interaction (2)	Topsmelt	133	40.8	< 0.0001	As above	Intercept
Embayment Distance from Guadalupe Interaction (1, 2)	Topsmelt	133	17.1	0.0007	As above	Intercept
Wetland (3)	Silverside	278	0.27	0.61 (NS)	Distance (-), Length (+), 2008 (-), 2009 (-), Distance*2009 (+)	Intercept, Length
Wetland (3)	Topsmelt	269	3.08	0.079 (NS)	Distance (-), Length (+)	Intercept, Length

Source: POTW 2008 Interaction (4)	Silverside	237	10.4	0.0012	Distance (-), Length (+), 2008 (-), 2009 (-), Distance*2009 (+), POTW (-), 2008*POTW (-)	Intercept, Length
Source: Contaminated Sediment (4)	Topsmelt	231	4.58	0.032	Distance (-), Length (+), 2008 (+), ContaminatedSediment (+), 2008*Distance (+)	Intercept

---

[a] Likelihood ratio tests were employed to answer the four study questions, on mixed models which account for additional significant predictor variables.

[b] Distance is always centered.

[c] Length = fish total length (centered).

Table 2. Forage fish Hg at mine sites and selected comparison sites

Site [a]	Mine influence	Species	Hg concentration Mean ± SD (N)
Guadalupe River upstream of Alviso Slough	New Almaden Mines	Silverside	0.16 ± 0.020 (4)
Study average (57 remaining sites)	Reference	Silverside	0.09 ± 0.059 (236)
Clear Lake [10]	Sulphur Bank Mine	Silverside	0.10 ± 0.055 (97) [b]
American Canyon Creek	Borges Mine	Topsmelt	0.030 ± 0.006 (4)
Study average (55 remaining sites)	Reference	Topsmelt	0.042 ± 0.020 (236)
Napa River	Bella Oaks Mine	Prickly sculpin	0.13 ± 0.009 (4)
Dry Creek	La Joya Mine	Prickly sculpin	0.068 ± 0.012 (4)
Clear Lake [10]	Sulphur Bank Mine	Prickly sculpin	0.13 ± 0.044 (5)
Sacramento-San Joaquin Rivers Delta [c]	Reference	Prickly sculpin	0.098 ± 0.062 (15) [d]
Napa River	Bella Oaks Mine	California roach	0.14 ± 0.003 (4)
Dry Creek	La Joya Mine	California roach	0.061 ± 0.003 (4)
Marsh Creek [43]	Mt. Diablo Mine	California roach	0.27 ± 0.21 (6)
California statewide [c]	Reference	California roach	0.084 ± 0.037 (45) [e]

Guadalupe River upstream of Alviso Slough	New Almaden Mines	Three-spined stickleback	0.30 ± 0.027 (4)
Dry Creek	La Joya Mine	Three-spined stickleback	0.099 ± 0.005 (2)
Zone 4 Line A	Industrial watershed	Three-spined stickleback	0.052 ± 0.004 (4)
Four additional Bay sites	Reference	Three-spined stickleback	0.096 ± 0.034 (11)
Marsh Creek [43]	Mt. Diablo Mine	Three-spined stickleback	0.082 ± 0.021 (6)
Putah Creek, CA Central Valley [c]	Reference	Three-spined stickleback	0.065 ± 0.007 (2) [f]
Walker Creek [44]	Gambonini Mine	Three-spined stickleback	0.19 (1) [g]

---

[a] Data were from the present study or other referenced studies, where noted.

[b] Whole body samples, collected 1999 to 2004

[c] www.ceden.us data query, March 23, 2013

[d] Average of site averages for eight sites, sampled by DG Slotton in 1998

[e] Average of site averages for eight sites, sampled 1995 to 1997

[f] Average of site averages for 26 sites, sampled 1991 to 2001

[g] Single composite of 36 individuals, collected June, 1992

Figure 1. Site average Hg concentrations in San Francisco Bay forage fish. a. Mississippi silverside. b. Topsmelt.

Figure 2. Hg concentrations in Mississippi silverside, as a function of distance from Guadalupe River, sampling year, and site category. Each point represents a composite sample, and lines represent linear model fits to the associated data type for the given year. POTW sites (i.e., draining wastewater treatment plants) (◆); all other sites (○).

Figure 3. Hg concentrations in topsmelt, as a function of distance from Guadalupe River and embayment category. Each point represents a composite sample, and lines represent linear model fits to the associated data type. Embayment sites (●); open sites (◆).

Figure 4. Hg concentrations in topsmelt, as a function of distance from Guadalupe River and site category. Each point represents a composite sample, and lines represent linear model fits to the associated data type. Contaminated sediment sites (◆); all other sites (○).

Figure 1a.

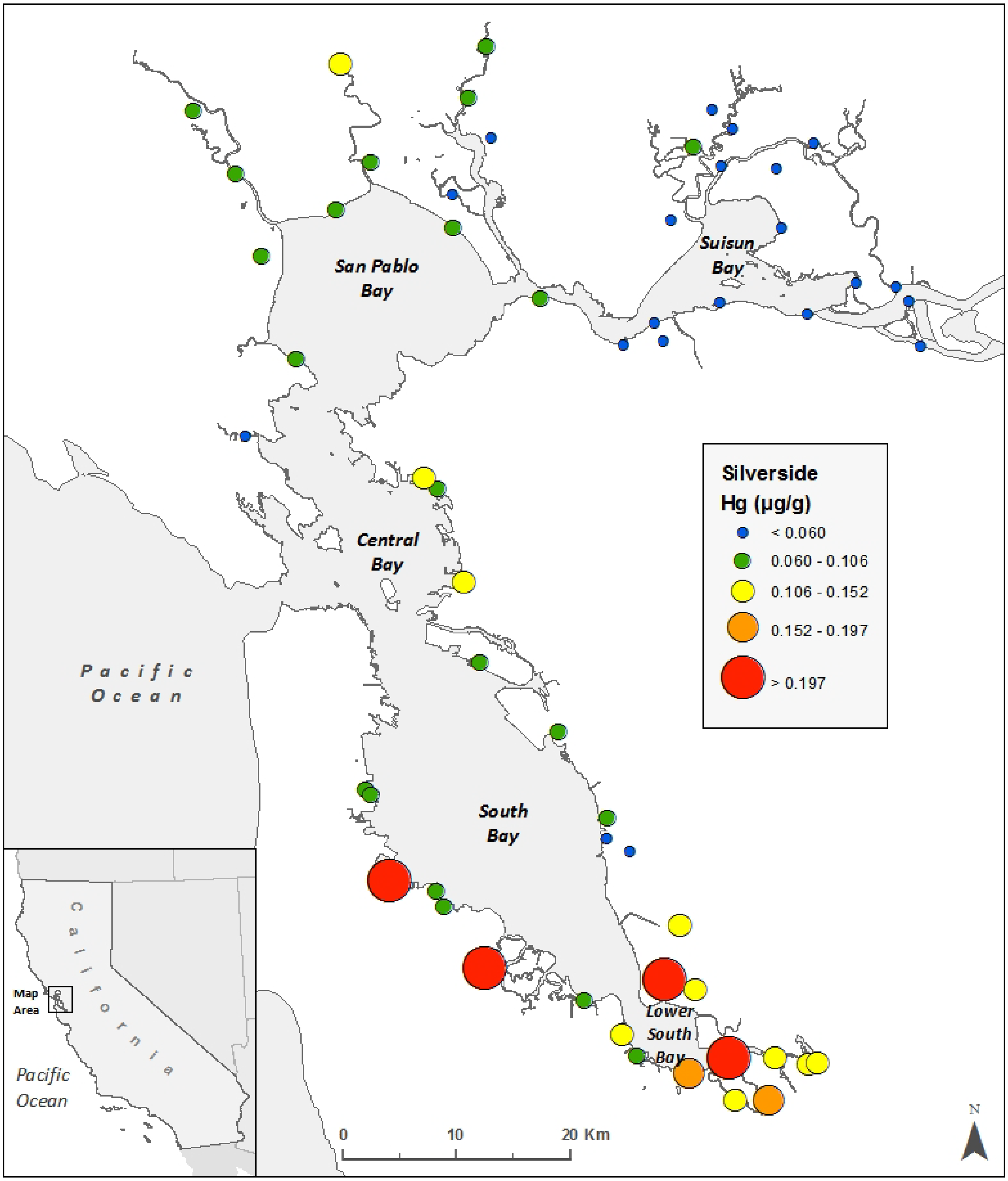
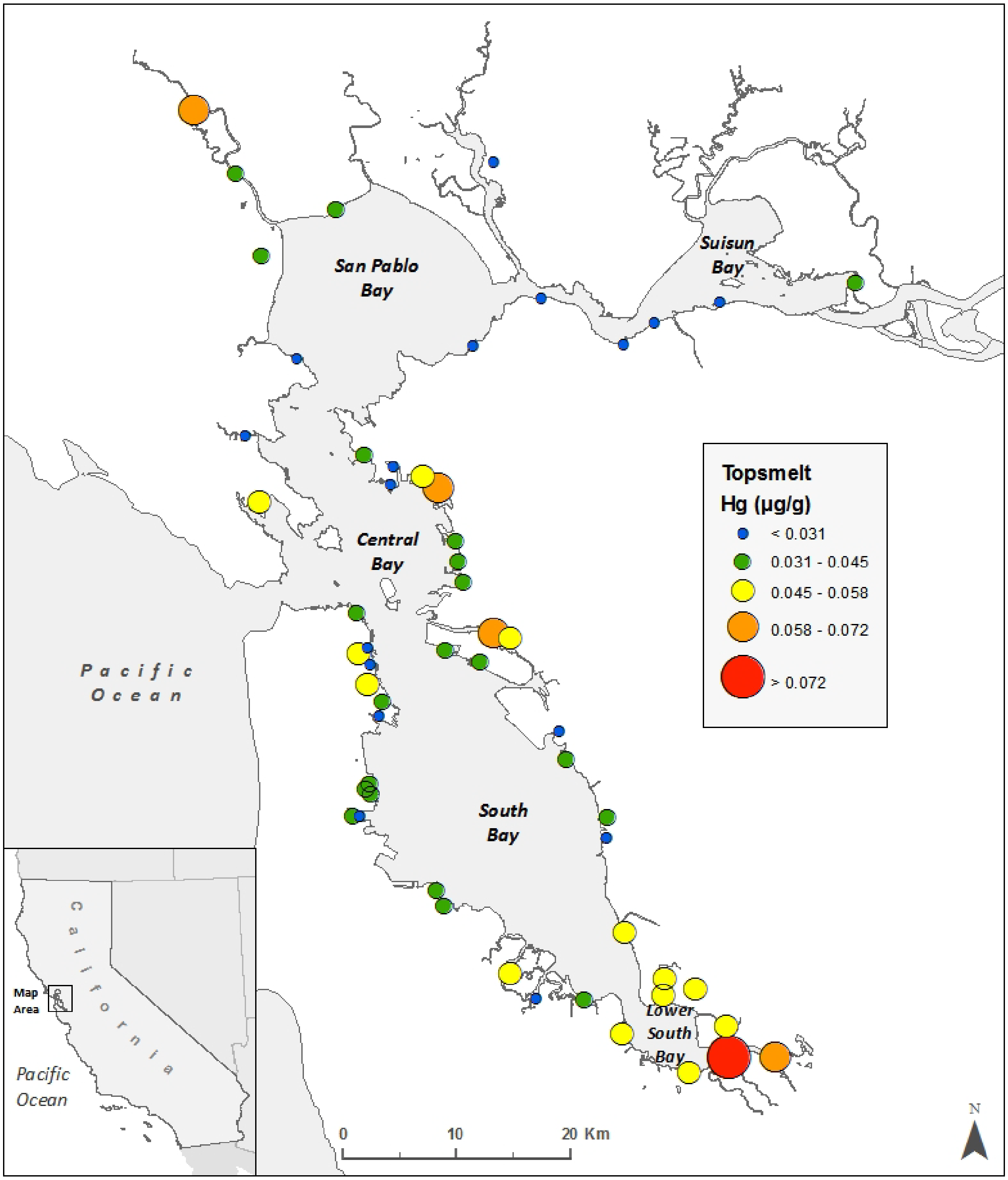

San Pablo Bay
Suisun Bay
Central Bay
South Bay
Lower South Bay
Pacific Ocean
Silverside Hg (µg/g)
< 0.060
0.060 - 0.106
0.106 - 0.152
0.152 - 0.197
> 0.197
California
Map Area
Pacific Ocean
0
10
20 Km
N

Figure 1b.



San Pablo Bay
Suisun Bay
Central Bay
South Bay
Lower South Bay
Pacific Ocean
Topsmelt Hg (µg/g)
< 0.031
0.031 - 0.045
0.045 - 0.058
0.058 - 0.072
> 0.072
California
Map Area
Pacific Ocean
0
10
20 Km
N

Figure 2.

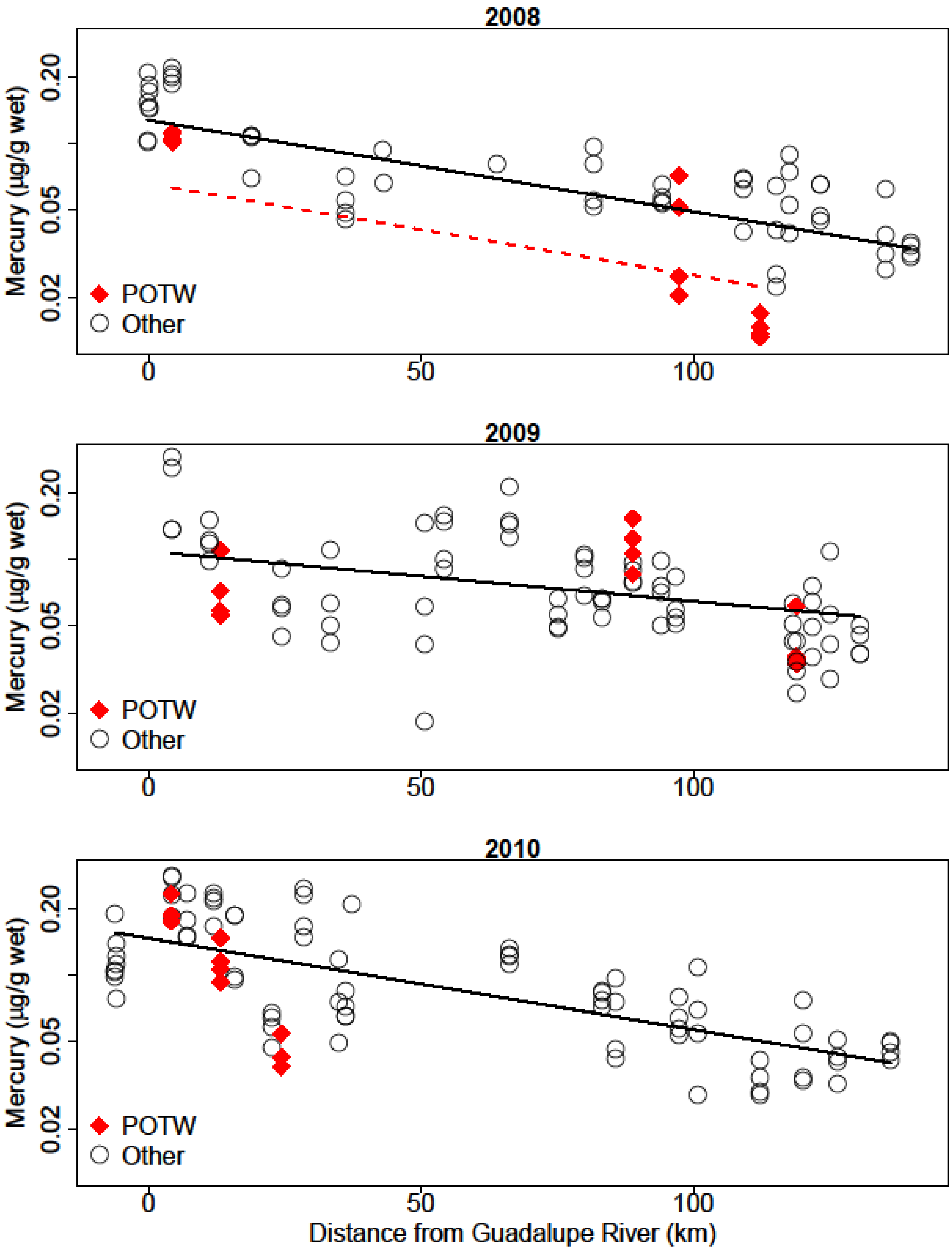

2008
Mercury (µg/g wet)
0.20
0.05
0.02
◆ POTW
○ Other
0
50
100
2009
Mercury (µg/g wet)
0.20
0.05
0.02
◆ POTW
○ Other
0
50
100
2010
Mercury (µg/g wet)
0.20
0.05
0.02
◆ POTW
○ Other
0
50
100
Distance from Guadalupe River (km)

Figure 3.

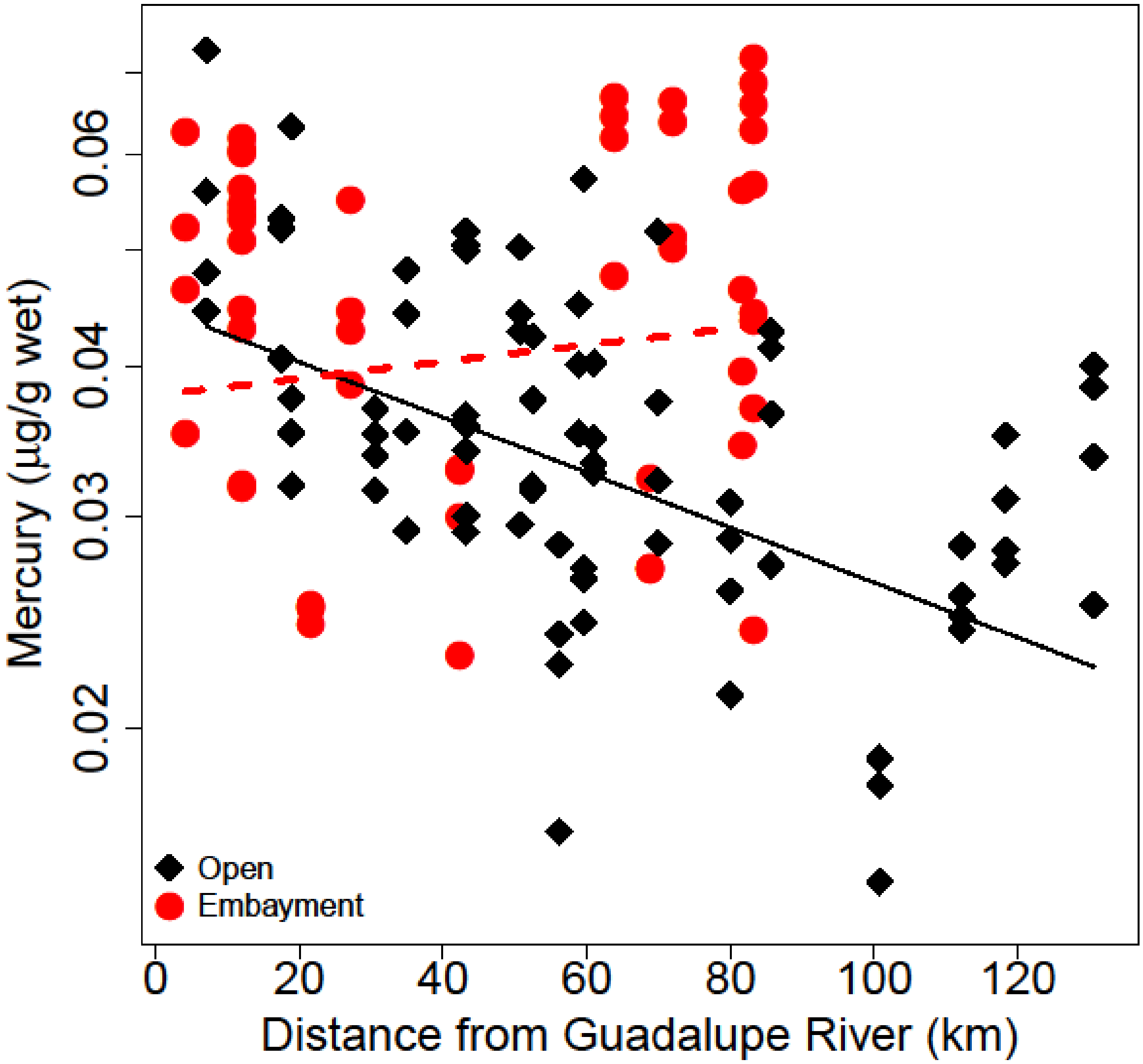
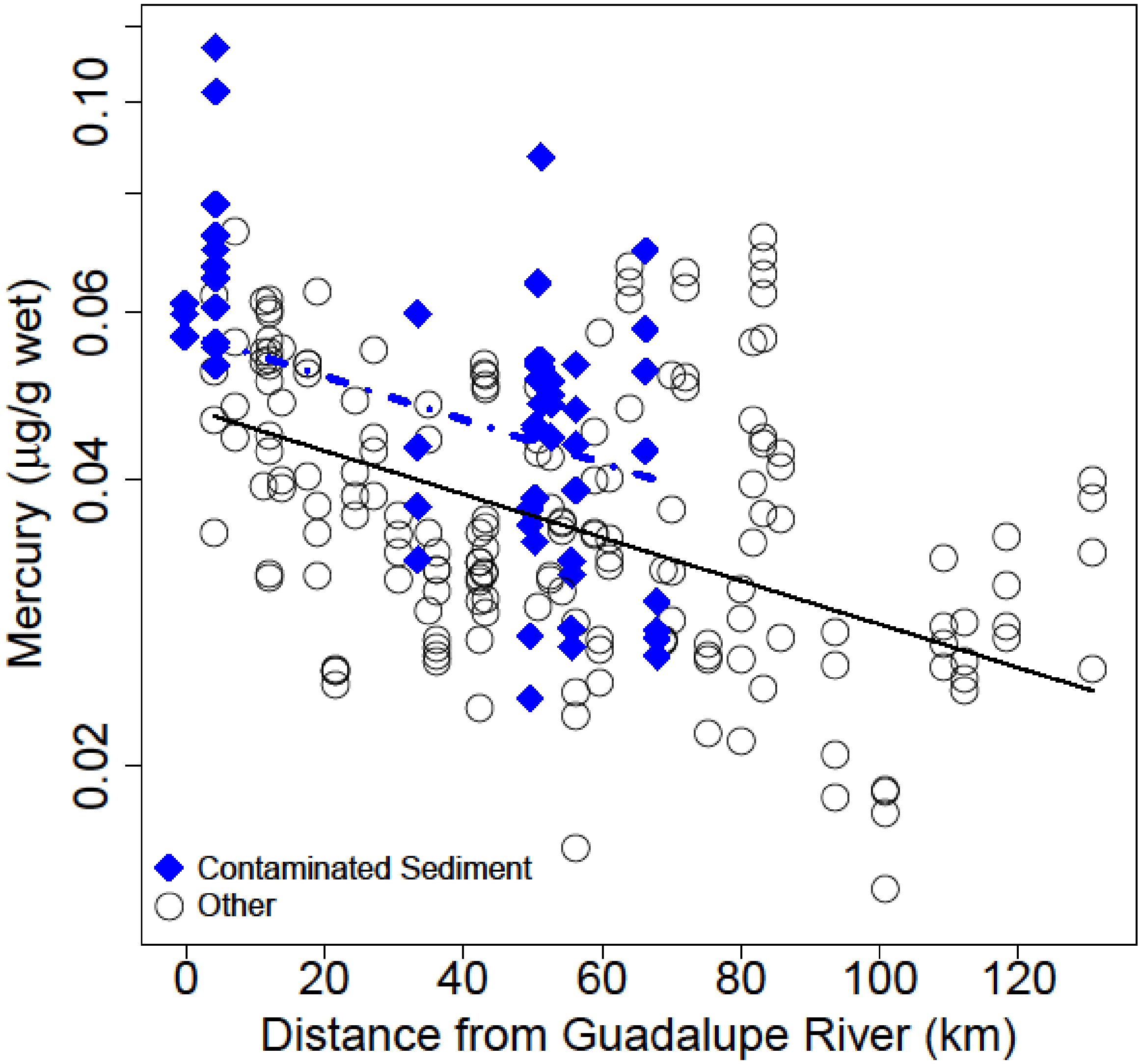

Mercury (µg/g wet)
0.02
0.03
0.04
0.06
0
20
40
60
80
100
120
Distance from Guadalupe River (km)
◆ Open
● Embayment

Figure 4.


Mercury (µg/g wet)
0.02
0.04
0.06
0.10
0
20
40
60
80
100
120
Distance from Guadalupe River (km)
◆ Contaminated Sediment
○ Other